Allium sativum, Nigella sativum, Caryophyllus dianthus and Punicagranatum in improvementof salmonellosis in experimental rabbit model.

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Abstract

The activity of medicinal plants against induced salmonellosis in rabbit was determined in vitro by Kirby-Baur , minimal inhibitory concentration (MIC) method . the results revealed that all the aqueous extract of the tested medicinal plants at a stock concentration of 2000 ng/ml had antisalmonella effect except Dianthus caryophyllus. Nigella sativum extract had the most potent effect with MIC (125 ng/ml)& both Allium sativum & punica granatum had MIC (250 ng/ml). In the mean time, a significant potentiation was obtained by double combination of chloramphenicol with Allium sativum or Punic granatum , MIC (31.25 ng/ml) or with Nigella sativum MIC (15.62 ng /ml). In conclusion both Allium sativum & Nigella sativum had shown antisalmonella activity when used singly or in combination with antibiotics.

الثوم ، الحبة السوداء، القرنفل وحب الرمان في تحسين النموذج التجريبي لمرض التيفوئيد في الارانب

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الخلاصة

ان فعالية النباتات الطبية المضادة لمرض التيفوئيد المحدث عملياً في الارانب تم قياسها بطريقة كيربي باور _ التركيز المثبط الاصغر _ بالزجاج حيث كشفت النتائج بان المستخلص المائي لجميع هذه النباتات المستعملة في هذه الدراسة له فعالية مضادة للتيفوئيد عندما استعملت بتركيز قياسي (٢٠٠٠) مايكروغرام /مل ماعدا مستخلص القرنفل .ان الخلاصة المائية للحبة السوداء كانت الاقوى حيث امتلكت تركيزاً مثبطاً اصغر يعادل (١٢٥)مايكرو غرام /مل في حين امتلك كل من الثوم وحب الرمان في خلاصتهم المائية تركيزاً مثبطاً اصغر يعادل (٢٥٠) مايكرو غرام/ مل وفي الوقت الذي حصلت فيه تقوية معتده عند الاتحاد الثنائي مابين الكلورامفينيكول والثوم او مع حب الرمان حيث كان التركيز المثبط الاصغر لاتحادهمايعادل (٣١,٢٥) مايكرو غرام / مل فان الاتحاد الثانئي للكلورامفينيكول مع الحبة السوداء كان الاقوى وهو اكبر من الثوم وحب الرمان وامتلك تركيزاًمثبطاً اصغر يعادل (١٥,٦٢) مايكرو غرام / مل .

الاستنتاج : امكانية استعمال الخلاصة المائية للثوم والحبة السوداء كوسيلة علاجية مساعدةللمرض التيفوئيد بدون ان تسبب اعراضاً جانبية وكذلك تقليلاً للنفقات

الكلمة المفتاح : الثوم ، الحبة السوداء ، مرض التيفوئيد ، التركيز المثبط الاصغر

Introduction

salmonellosis is an infection disease caused by any strain of the genus salmonellae . the clinical features of the disease include fever , headache , drowsiness , malaise , anorexia , diarrhea or constipation , relative bradycardia and splenomegaly[1]. Rabbit is a good model for study of salmonellosis by producing many lesions in the intestine during salmonella typhi including edema, hemorrhage , ulceration and enlarged follicles[2]. A new research reported that the extract of punica granatum had antisalmonella activity in experimental rabbit model of salmonellosis[3]. The current study was carried out to examine the possible antisalmonella activity of Allium sativum (garlic), Nigella sativum (black cumin) and caryophyllus dianthus (carnation) in comparison with punica granatum (pomegranate) which was used as a control in order to find a safe , cheap & more efficacious therapy.

Material and methods

The antisalmonella activity of the medicinal plants was determined by MIC method (in vitro) or by induction of salmonellosis in rabbit (in vivo) but this article deals with in vitro assessment only.

Chemicals

The antimicrobial drugs used in the present study include chloramphenicol (miphencol-Misr comp), amoxicillin (panpharma-France) ciprofloxacin (ciprodar-Kimadia-Jordan) trimethoprim-sulfamethoxazole (methaprim- SDI- Iraq).

Plant extraction

The bulb of garlic seed of black cumin, fruit of carnation, seed of pomegranate were purchased from well known bureau (Al-Medina) in Baghdad city. The plants were identified and authenticated by Iraq national institute for herbs. These dried plants were cleaned carefully and powdered with an electrical grinder then passed through sieve no 4 to remove the debris . The sieved powder was store in air tight, black container at room temperature. The aqueous extract was prepared by diluting on volume of well grinded powder to 10 volume of water at 80 C^0 in the stoppered flask after shaking completely , thus it was allowed to stand for 10 minutes to be cold and filtered for practical use . The aqueous extract should be used within 12 hours[(4)]. The plant extract were diluted so that a stock concentration of 2000 ng /ml was obtained and then serially diluted with two-fold dilution for 7 sequenced dilution with a Mueller-Hinton broth. Equal doses of 0.1ml salmonella typhi was added to the tubes and mixed well followed by cultivation 24 hours in incubation at 37 C^0 (the inoculums was performed according to Macferland of international standard) in order to observe turbidty appearance. The obtained results were collected for analysis and assessment.

Results and discussion

The diagnosis of salmonellosis is confirmed by eliciting positive samples of blood, urine and stool culture ,however widal test of increasing titer over a week is also significant[5] in addition to the clinical features. Salmonellae are theoretically susceptible to antimicrobials that show cidal effect against gram negative bacteria such as chloramphenicol [6]. Indeed, the antisalmonella effect of the tested medical plants, showed no significant difference over the results of 75% which was related to their inhibitory action on gram negative bacteria [7] at $p<0.05$.

The results of this study showed that aqueous extract of nigellsativum had the most potent inhibitory action against s.typhi with MIC (125ng\ml) where as Dianthus caryophyllus had negative invitro effect. In the mean time both Allium sativum and punica granatum had MIC (250 ng\ml) table 1 these results are compatible with the results of others [8]. Who showed that the root of diospyrus piscatoia had antisalmonella activity with MIC (25-100 ng\ml).

The combination of plant extract , with antimicrobials drugs had shown a masked inhibition of s.typhi by Nigella sativum , allium sativum and punica granalum at stock concentration of 1000 ng\ml as well. The highest potency was recorded for combination of Nigella sativum extract with chloramphenicol (MIC 15.62 ng\ml) as compared with Nigella sativum alone MIC (125 ng\ml) . in the mean time chloramphenicol plus Allium sativum or punica granalum extract came secondly MIC (1.25 ng\ml) for each.(table -2). MIC test confirmed the equipotent effect of both Allium Sativum and punica granatum to those of the corresponding antimicrobials namely amoxicillin, ciprofloxacin and trimethoprim –sulfa methoxazole(table -2).

The antisalmonella synergism that observed after combination of Allium sativum , Nigella sativum or punica granatum with antimicrobial chloramphinicol , amoxicillin , ciprofloxacin and trimethoprim could be attributed to special mechanism of action than the common mechanism of synergism. This point can possibly promise to minimize the rate of drug resistance.

Finally, in shedding light on the obtained results, the medicinal plant allium sativum, nigella sativum could be a good remedy for the treatment of induced salmonellosis without causing any important adverse effects in addition to reduction of their financial cost.

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Table (1) shows antisalmonella MIC values of the aqeous extracts 0f 4.

Medicinal plants			Inhibitoryactivity Against salmonella							
latin name	Common name	Medi c-inal part	No. of tube	1	2	3	4	5	6	7
			dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128
			Concentrat-ion mg\ml	1000	500	250	125	62.5	31.25	15.62
Distilledwater	-	-		-	-	-	-	-	-	-
Allium sativum	Garlic	Dried bulb		+	+	+	-	-	-	-
Dianthus caryphyllus	carnation	Dried fruit		-	-	-	-	-	-	-
Nigella sativum	Black cumin	Dried seed		+	+	+	+	-	-	-
Punica granatum	pomegranate	Dried seed		+	+	+	_	_	_	_

Table (2) MIC tests of the double combination models between antimicrobials and medicinal plants extracts

Combined agents					Inhibitory activity against salmonella						
antimicrobial	stock	Medicinal plant	stock	No. of tube	1	2	3	4	5	6	7
	Halved dose		Halved dose	Dilution	1:2	1:4	1:8	1:16	1:32	1:64	1:128
	µg\ml		µg\ml	Concentration µg\ml	1000	500	250	125	62.5	31.25	15.2
Amoxicillin	1000	Allium sativum (garlic)	1000		+	+	+	+	+		
Amoxicillin	1000	Nigella sativum (black cumin)	1000		+	+	+	+	+		
Amoxicillin	1000	Punica granatum (pomegranate)	1000		+	+	+	+	+		
Chloramphenicol	1000	Allium sativum (garlic)	1000		+	+	+	+	+	+	
Chloramphenicol	1000	Nigella sativum (black cumin)	1000		+	+	+	+	+	+	+
Chloramphenicol	1000	Punica granatum (pomegranate)	1000		+	+	+	+	+	+	
Trimethoprim-sulfamethoxazole	1000	Allium sativum (garlic)	1000		+	+	+	+	+		
Trimethoprim-sulfameth	1000	Nigella sativum (black	1000		+	+	+	+	+		

oxazole		cumin)									
Trimethoprim-sulfamethoxazole	1000	Punica granatum(pomegranate)	1000		+	+	+	+	+		
ciprofloxacin	1000	Allium sativum (garlic)	1000		+	+	+	+			
ciprofloxacin	1000	Nigella sativum (black cumin)	1000		+	+	+	+	+		
ciprofloxacin	1000	Punica granatum(pomegranate)	1000		+	+	+	+			